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(54) Title: WOUND HEALING AND TREATMENT OF FIBROTIC DISORDERS					
(57) Abstract					
A healing composition containing at least one non-fibrotic growth factor in combination with a pharmaceutically acceptable carrier is disclosed. A method of preparation of the composition and method of treating a host, suffering from a wound or fibrotic condition, disease, disorder with the composition is also disclosed.					

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WOUND HEALING AND TREATMENT OF FIBROTIC DISORDERS

This invention concerns the healing of wounds and other conditions in which fibrosis is a major mechanism of tissue repair or where excessive fibrosis leads to pathological derangement and malfunctioning of the tissues. It refers in particular to agents and techniques for facilitating repair and healing of animal tissues, without excessive fibrosis, and for preventing or treating diseases and conditions of fibrosis.

Fibrosis is a major problem in wound healing causing scarring of the tissue, which not only looks unsightly, but also causes problems in respect of growth of the tissue, function, movement etc. This is particularly true following injuries to children or following major burns.

In addition, fibrosis is a major medical problem where abnormal or excessive deposition of fibrous tissue occurs in many diseases, including liver cirrhosis, glomerulonephritis, pulmonary fibrosis, systemic fibrosis, rheumatoid arthritis, as well as wound healing.

The mechanism of fibrosis is still not fully understood, but wound healing usually begins as an

inflammatory reaction with leucocyte infiltration and accumulation of cytokines. These cytokines are responsible for the proliferation of fibroblasts and the deposition of extracellular matrix proteins (including collagen and fibronectin) which accumulate and result in permanent alteration in tissue structure and function.

Examples of the regulatory cytokines include tumor necrosis factor (TNF), fibroblast growth factors (FGF's), platelet derived growth factor (PDGF) and transforming growth factor β (TGF β), (TGF β -1 to TGF β -5 have so far been identified). Two of these cytokine families, TGF β and PDGF, have been reported to be highly fibrogenic, and, moreover, inhibition of two of the TGF β 's and PDGF activity, using anti-TGF β -1, anti-TGF β -2 and anti-PDGF antibodies, has been shown to diminish fibrosis in tissue injury (Shah et al, *The Lancet*, 339, 213-214, 1992; WO 91/04748).

The present invention provides novel compositions useful in the treatment of wounds and fibrotic disorders and which may prevent, inhibit or reverse fibrosis.

The invention comprises a healing composition containing at least one non-fibrotic growth factor in combination with a pharmaceutically acceptable carrier.

The composition may comprise TGF β -3 as the or a non-fibrotic growth factor, such that on application of the composition to the tissue, this non-fibrotic growth factor is present in an elevated level compared to its naturally occurring level.

The composition may comprise acidic or basic FGF as the or a non-fibrotic growth factor, again resulting in a much elevated level of non-fibrotic growth factor than would naturally be present.

The composition may comprise anti-fibrotic agents, such as fibrotic growth factor neutralising agents, for example antibodies to TGF β -1, TGF β -2 and PDGF; binding proteins which prevent TGF β -1, TGF β -2 and PDGF from binding to their receptors by either binding to the growth factor itself, eg. Decorin, Biglycan, or binding to the receptor, eg. peptides containing the receptor binding site sequence or soluble forms of the growth factor receptors and the growth factor binding domains of these receptors; and antisense oligonucleotides or ribosymes which act to prevent fibrotic growth factor mRNA translation.

The composition may comprise combinations of non-fibrotic growth factors, for example, TGF β -3 and anti-fibrotic agents, for example, anti-TGF β -1 and anti-TGF β -2.

The non-fibrotic growth factor and/or anti-fibrotic agent(s) may be present in the composition in an active or inactive form. Inactivation may be by

any of a number of mechanisms, for example, by encapsulation. Capsules may be degradeable by an external stimulus to release the active form when required. The external stimulus may include UV light, ultrasound, in vivo enzymes or heat.

Inactivation may, however, be by the molecular addition of a binding molecule. The binding molecule may be detachable when required by an external stimulus such as UV light, ultrasound, in vivo enzymes or heat.

The non-fibrotic growth factor may be present in an inactive form, for example, as a precursor, and may be activated upon contact with tissue containing the natural cleavage enzymes required to convert the precursor into its active form.

The carrier may comprise a neutral sterile cream, gel, aerosol or powder for topical application, or may be in the form of a patch, sterile dressing or an absorbable dressing. The carrier may be a biopolymer of collagen, hyaluronic acid or polymer of PVC to which the anti-fibrotic or non fibrotic agents are attached in such a way as to facilitate their action and/or release when the carrier is in contact with or implanted into either the wound or fibrotic lesion. The carrier may also comprise a sterile solution for irrigation,

injection either locally or systemically or inhalation, or may be in the form of a tablet, capsule, and the like, for enteral administration.

The present invention also provides a method of preparation of a pharmaceutical healing or anti-fibrotic composition containing at least one non-fibrotic growth factor for topical application in a cream, gel, aerosol, powder, patch, dressing, biopolymer or polymer implant, delay or slow release system, or in a solution for irrigation, injection or inhalation, or in a tablet or capsule for enteral administration.

The present invention also provides a method of inhibiting fibrosis during the healing of wounds and in other fibrotic conditions and disorders, for example ulcers, comprising administering to a host suffering from tissue wounding or other fibrotic conditions and disorders, at least one non-fibrotic growth factor.

The present invention also provides a method of reversing fibrosis in such fibrotic conditions and disorders comprising administering to a host suffering from such fibrotic conditions and disorders, at least one non-fibrotic growth factor, for example, TGF β -3 and/or at least one anti-fibrotic agent for example, anti-TGF β -1/TGF β -2.

As mentioned above, two cytokines have been identified as being involved in fibrosis, namely PDGF and TGF β . Of these two, TGF β appears to play the major role. For example, in tissues which heal without scar formation, such as fetal and embryonic wounds where there is a lowered inflammatory response and altered cytokine profile, the level of TGF β in particular, is much reduced.

TGF β comprises a family of molecules, the important mammalian members being TGF β -1, TGF β -2 and TGF β -3 (Roberts and Sporn, The Transforming Growth Factor- β s, In: Peptide Growth Factors and their Receptors, Springer Verlag, Berlin, 1990, p418-472). The TGF β s, although having different patterns of expression, share over 70% peptide homology and are thought to have similar functions and act interchangeably. Thus in wound healing it would be expected that TGF β -3 would act like TGF β -1 and TGF β -2 to increase extracellular matrix production, angiogenesis and the inflammatory response.

As discussed above, fibrotic disease is a major medical problem. In such diseases, there is abnormal or excessive deposition of fibrous tissue. Such diseases are exemplified by liver cirrhosis, glomerulonephritis, pulmonary fibrosis, systemic fibrosis and rheumatoid

arthritis. In such diseases the use of TGF β would be avoided, since TGF β 's are believed to increase the deposition of fibrous tissue. Surprisingly, it has now been discovered that TGF β -3 has the opposite effect to that expected, in that it promotes healing without promoting the deposition of fibrous tissue.

The present invention provides the use of a TGF β -3 for the manufacture of a medicament for the treatment of a fibrotic disease.

The present invention also provides a method of treating a fibrotic disease by administering a pharmaceutically effective amount of TGF β -3 to a patient in need thereof.

The present invention also provides an agent for treating a fibrotic disease which comprises TGF β -3 as active ingredient.

The present invention also provides a pharmaceutical composition comprising a higher proportion of TGF β -3 in relation to TGF β -1 or TGF β -2, compared with relative proportions in naturally occurring TGF β , and a pharmaceutically acceptable carrier.

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EP 0 433 225 defines the biological activity of the TGF β 's ie. TGF β -1, TGF β -2 and TGF β -3, as including the ability to increase formation of fibrous granular tissue in and around wound implants in rats (page 5, lines 17-19), while US 4,810,691 and US 4,774,228 describe the use of TGF β 's for promoting connective tissue deposition.

Experiments described in detail below indicate that contrary to the conventional view that TGF β -3 acts in the same manner as TGF β -1 and TGF β -2 to increase fibrosis at the site of wound healing, it has in fact the opposite effect and promotes wound healing with reduced fibrosis and scarring.

Experiments

The experiments have involved exogenous injection of TGF β -1, TGF β -2 or TGF β -3. They have also involved the injection of neutralising antibodies to TGF β -1 or TGF β -2 (or anti TGF β -1 and TGF β -2 in combination). Neutralising antibodies to TGF β -3 are not yet available. The experimental protocol was as described in Shah et al, The Lancet, 339, 213-214, 1992)

These experiments produced a very interesting and unexpected set of results. First, the neutralising

antibody to TGF β -1 diminished scarring, i.e. reduced the amount of extracellular matrix, reduced angiogenesis and reduced the numbers of macrophages and monocytes at the wound. It also improved the orientation of collagen fibres in the healing wound. The neutralising antibody to TGF β -2 had very little effect on its own, but showed a slight improvement in scarring. Combined, the neutralising antibodies to TGF β -1 and TGF β -2 showed a marked improvement in wound healing (similar to that described in Shah et al, *The Lancet*, 339, 213-214, 1992), namely decreased extracellular matrix deposition (decreased fibronectin, decreased collagen), decreased angiogenesis, decreased macrophages and monocytes at the wound site and better orientation of collagen and fibronectin within the wound. Exogenous addition of TGF β -1 or TGF β -2 had the expected result, namely of increasing extracellular matrix deposition and angiogenesis. However, exogenous addition of TGF β -3 did not have this effect, but rather produced effects similar to those observed with the neutralising antibodies to TGF β -1 and TGF β -2, namely, a reduction in the amount of extracellular matrix deposited, a decrease in macrophages and monocytes and a marked improvement in subsequent scarring.

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Specific details of the experiments to document the TGF β -3 effect are as follows :-

Adult male Sprague-Dawley rats (200 to 250 gram weight) were anaesthetised with halothane nitrous oxide and oxygen inhalation. Two incisions, ten millimetres in length and to the depth of the parnicalous carnosis were made in the dorsal skin, equal distant from the midline and between the fore and hind limbs. The wounds were left unsutured to heal by secondary intention to produce the greatest amount of granulation tissue and scarring. In each animal, one wound (control) was unmanipulated. In different animals the other wound received a) an injection of transforming growth factor beta 1 (TGF β -1) (20 ng per injection), or b) an injection of TGF β -2 (20 ng per injection) or c) an injection of TGF β -3 (20 ng per injection). It had previously been determined from dose response experiments that 20 ng per injection was the optimum dose to give. Injections were of 100 microlitres in phosphate buffered saline and were introduced into the wound margins by local infiltration on days 0, 1 and 2. The fluid was infiltrated along the length of each wound margin through a single entry point 0.5 cm distal to the caudal end of the wound. At least four animals were killed by chloroform overdose on each of days 7, 14 and 42 after wounding. The wounds were processed for

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routine histological examination, particularly using connective tissue stains such as Mallory or Masson's trichrome. They were also processed for immuno-cytochemistry, using antibodies to detect fibronectin (as a marker of early wound repair and to show the orientation of extracellular matrix molecules), macrophages and monocytes (as an indication of the inflammatory response), laminin (to highlight basement membranes, e.g. of newly formed blood vessels) and collagen types I and III to document connective tissue deposition within the wound and scarring.

Summary of Results

Compared to control wounds, at 7 and 14 days, the TGF β -3 treated wounds had less fibronectin and the fibronectin fibres were in a better orientation. By six weeks, the fibronectin in all wounds was similar in quantity to that of the surrounding normal skin. However, that in the TGF β -3 treated wound had a much better orientation than the other wounds. The results were almost indistinguishable from the results obtained with neutralising antibodies to TGF β -1 and TGF β -2. By comparison, wounds treated with TGF β -1 or TGF β -2 showed a vastly increased quantity of fibronectin in the wound at 7 days and this fibronectin had an abnormal orientation, compared to the surrounding tissue. The

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same was true at 14 days, but by 6 weeks there was little difference between the TGF β -1 or TGF β -2 treated wounds and the control in terms of the quantity of fibronectin present.

At 7 days TGF β -1 treated and TGF β -2 treated and control wounds showed similar profiles of macrophage and monocyte infiltration (for example control 159, TGF β -1 149, control 117, TGF β -2 112 per section). However, TGF β -3 treated wounds had a low profile of macrophage plus monocyte infiltration (control 130, TGF β -3 91 per section).

At 7 days TGF β -1 treated and TGF β -2 treated wounds had a higher profile of macrophages in the lower half of the wounds compared to similar areas in the control wounds (control 50/TGF β -1 80, control 45/ TGF β -2 59 per section). However, in the upper half of the wounds the macrophage infiltration was similar in the TGF β -1 treated and control wounds (control 37, TGF β -1 39 per section) whilst TGF β -2 treated wounds had a lower profile (control 34, TGF β -2 19). By contrast, TGF β -3 treated wounds showed a lower macrophage profile throughout the entire wound, compared to the control wounds (upper half control 41, TGF β -3 16; lower half control 72, TGF β -3 28 per section).

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Laminin staining was used as a marker of neovascularisation. At 7 days, TGF β -1 treated wounds showed an increase in the number of blood vessels, particularly at the base of the wound. TGF β -2 treated wounds appeared similar to the control wounds. TGF β -3 treated wounds, however, had many more blood vessels compared to either the control or the TGF β -1 or the TGF β -2 treated wounds. This was a very marked effect.

By 14 days there were few differences in the number of blood vessels between either the TGF β -1, TGF β -2 or TGF β -3 treated wounds compared to the control. However, the TGF β -3 treated wounds tended to have more blood vessels.

In terms of collagen deposition within the wound, as assayed by Mallory staining or immunocyto-chemistry, treatment of the wound with either TGF β -1 or TGF β -2 increased the amount of collagen within the wound on days 7 and 14 after wounding. Furthermore, this collagen had an abnormal orientation with a much higher percentage of fibres orientated in a vertical direction, compared to the surrounding dermis. At six weeks, the control, TGF β -1 and TGF β -2 treated wounds were visibly scarred with an abnormal accumulation of abnormally orientated collagen within the wounded area. By contrast, wounds treated with TGF β -3 showed slightly

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less collagen deposition on days 7 and 14 after wounding. Moreover, the collagen deposited was in a similar reticular pattern to that of the surrounding dermis. Consequently, by six weeks after wounding, the TGF β -3 treated wounds had a more similar dermal architecture to that of the surrounding normal skin, compared to either the control TGF β -1 or TGF β -2 treated wounds. This result with TGF β -3 is very similar to that obtained with neutralising antibodies to TGF β -1 and TGF β -2.

In summary, therefore, treatment of the wounds with TGF β -3 decreased the amount of extracellular matrix deposited in the early wound, assured that the orientation of this matrix was in the normal reticular pattern of the dermis, compared to the abnormal pattern of the scar, decreased the number of macrophages and monocytes and hence inflammatory infiltrate into the wound, but greatly increased the number of blood vessels in the early healing wound. These effects are almost identical to those observed with neutralising antibodies to TGF β -1 and TGF β -2 except the increase in the number of blood vessels. Treatment of the wounds with neutralising antibodies to TGF β -1 and TGF β -2 decrease the amount of extracellular matrix deposited, alter the orientation of this matrix, so that it is in a more normal alignment, decrease the inflammatory infiltrate

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of macrophages and monocytes (like TGF β -3) but decrease the number of blood vessels (unlike treatment with TGF β -3 which increases the number of blood vessels).

TGF β -3 therefore acts as an anti-scarring (anti-fibrotic) agent. It is very clear that this is an isoform specific effect within the TGF β family.

TGF β -3 therefore becomes a target as an anti-fibrotic agent or an anti-scarring agent. It may be capable of biological modification to increase the anti-fibrotic effect or define more carefully that portion of the molecule responsible for these effects. It may be possible to optimise the structure of TGF β -3 as an anti-fibrotic agent, based on such analysis. The effects of TGF β -3 in this regard are unpredictable from the literature, and interestingly, differ from the neutralising antibody experiments, particularly in the increase in angiogenesis. This may actually be beneficial for certain kinds of wound healing, e.g. chronic wounds such as venous leg ulcers, where one wants to increase the vascular supply to stimulate healing but decrease subsequent scarring.

In the context of fibrosis, the effects of TGF β -3 or anti TGF β -1/TGF β -2 agents are not limited to preventing further increases of fibrosis. TGF β -1/TGF β -2

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act to increase the accumulation of extracellular matrix molecules both by stimulating synthesis of new extracellular matrix molecules and decreasing the removal of existing matrix molecules, i.e. inhibiting tissue turnover (Roberts and Sporn, the transforming growth factor - β 's, In: Peptide growth factors and their receptors, Springer Verlag, Berlin, 1990, p 418-472). Therefore, any agent which antagonises or neutralises or renders ineffective TGF β -1/TGF β -2 not only decreases extracellular matrix synthesis but also increases remodelling. As an anti-fibrotic agent either TGF β -3 or anti-TGF β -1/anti- TGF β -2/anti-PDGF (or some combination thereof) may in certain fibrotic diseases, e.g. glomerulonephritis, pulmonary fibrosis, reverse the accumulation of fibrous scar tissue already present in the tissue.

It will be appreciated that it is not intended to limit the invention to the above examples only, many variations, such as might readily occur to one skilled in the art, being possible, without departing from the scope thereof as defined in the appended claims.

Thus for example, as well as applying a preparation to a wound containing TGF β -3 only, this may be given in combination with fibrotic growth factor neutralising agent(s), for example, anti-TGF β -1 and/or

anti-TGF β -2 and/or anti-PDGF antibodies, in a ratio which will enable the required amount of vascularisation for the particular type of wound to be provided whilst at the same time healing the wound without scarring.

CLAIMS

1. A healing composition containing at least one non-fibrotic growth factor in combination with a pharmaceutically acceptable carrier.
2. A composition according to claim 1, wherein the non-fibrotic growth factor comprises TGF β -3.
3. A composition according to claim 1 or claim 2, wherein the non-fibrotic growth factor comprises FGF.
4. A composition according to any preceding claim, comprising anti-fibrotic agents.
5. A composition according to claim 4, wherein the anti-fibrotic agents include antibodies to TGF β -1, TGF β -2 and PDGF; binding proteins which prevent TGF β -1, TGF β -2 and PDGF from binding to their receptors by either binding to the growth factor itself, eg. Decorin, Biglycan, or binding to the receptor, eg. peptides containing the receptor binding site sequence; or soluble forms of growth factor receptor or the growth factor binding domains of these receptors or antisense oligonucleotides or ribosymes which act to prevent fibrotic growth factor mRNA translation.

6. A composition according to any preceding claim wherein the non-fibrotic growth factor and/or anti-fibrotic agent(s) are present in the composition in an active form.
7. A composition according to any of claims 1 to 5, wherein the non-fibrotic growth factor and/or anti-fibrotic agent(s) are present in the composition in an inactive form.
8. A composition according to claim 7, wherein inactivation is by encapsulation.
9. A composition according to claim 8, wherein the capsules are degradable by an external stimulus to release the active form when required.
10. A composition according to claim 9, wherein the external stimulus includes UV light, ultrasound, in vivo enzymes or heat.
11. A composition according to claim 7, wherein inactivation is by the molecular addition of a binding molecule which is detachable when required by an external stimulus including UV light, ultrasound, in vivo enzymes or heat.

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12. A composition according to any preceding claim, wherein the non-fibrotic growth factor is present in an inactive form, for example, as a precursor, and is activated upon contact with tissue containing the natural cleavage enzymes required to convert the precursor into its active form.
13. A composition according to claim 1, wherein the carrier comprises a neutral sterile cream, gel, aerosol or powder for topical application.
14. A composition according to claim 1, wherein the carrier comprises a patch or a sterile dressing or an absorbable dressing for topically covering a wound.
15. A composition according to claim 1, wherein the carrier comprises a sterile solution for irrigation, injection or inhalation.
16. A composition according to claim 1, wherein the carrier comprises a tablet, capsule, and the like, for enteral administration.
17. A composition according to claim 1, wherein the carrier comprises a biopolymer, for example collagen, hyaluronic acid or polymer, for contacting or implanting into the wound/fibrotic lesion so as to allow release of

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the active agents slowly or quickly and for to be active in situ.

18. A method of preparation of a pharmaceutical healing or anti-fibrotic composition containing at least one non-fibrotic growth factor for topical application in a cream, gel, powder, aerosol, patch or dressing, biopolymer or polymer implant, delay or slow release system or in a solution for irrigation, injection or inhalation, or in a tablet or capsule for enteral administration.

19. A method of inhibiting fibrosis during the healing of wounds and other fibrotic diseases, disorders or conditions, comprising administering to a host suffering from tissue wounding or these fibrotic conditions, at least one non-fibrotic growth factor.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 93/00586

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)⁶

According to International Patent Classification (IPC) or to both National Classification and IPC
 Int.C1. 5 A61K37/02; A61K39/395; C07K15/00

II. FIELDS SEARCHED

Minimum Documentation Searched⁷

Classification System	Classification Symbols
Int.C1. 5	A61K ; C07K

Documentation Searched other than Minimum Documentation
 to the Extent that such Documents are Included in the Fields Searched⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X,P	WO,A,9 217 206 (THE VICTORIA UNIVERSITY OF MANCHESTER) 15 October 1992 see page 4, line 11 - page 13, line 23 ---	1,3,4-19
X	WO,A,9 003 810 (ED GEISTLICH SÖHNE AG FÜR CHEMISCHE INDUSTRIE) 19 April 1990 see page 1, line 1 - line 19 ---	1,3
X	EP,A,0 375 127 (GENENTECH) 27 June 1990 see column 5, line 41 - line 51 see column 7, line 21 - column 12, line 16 ---	1,2,6-19
		-/-

⁶ Special categories of cited documents :¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

14 JULY 1993

Date of Mailing of this International Search Report

23.07.93

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

REMPP G.L.E.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	WO,A,9 110 727 (LA JOLLA CANCER RESEARCH FOUNDATION) 25 July 1991 see page 10, line 25 - page 11, line 11 see page 22, line 6 - page 23, line 21 -----	1,4,5
X	EP,A,0 433 225 (CIBA-GEIGY AG) 19 June 1991 cited in the application see page 4, line 29 - page 5, line 19 see page 8, line 22 - page 9, line 39 -----	1,2,6, 13-19

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

GB 9300586
SA 72604

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9217206	15-10-92	AU-A-	1436892	02-11-92
WO-A-9003810	19-04-90	None		
EP-A-0375127	27-06-90	AU-A-	4524889	28-05-90
		CA-A-	2002130	02-05-90
		WO-A-	9004974	17-05-90
WO-A-9110727	25-07-91	None		
EP-A-0433225	19-06-91	AU-A-	6701890	13-06-91
		JP-A-	3191791	21-08-91

INTERNATIONAL SEARCH REPORT

rnational application No.

PCT/GB93/00586

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 19 is directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.